

UCbase & miRfunc: a database of ultraconserved sequences and microRNA function

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Received July 9, 2008; Revised September 3, 2008; Accepted September 29, 2008

ABSTRACT

Four hundred and eighty-one ultraconserved sequences (UCRs) longer than 200 bases were discovered in the genomes of human, mouse and rat. These are DNA sequences showing 100% identity among the three species. UCRs are frequently located at genomic regions involved in cancer, differentially expressed in human leukemias and carcinomas and in some instances regulated by microRNAs (miRNAs). Here we present UCbase & miRfunc, the first database which provides ultraconserved sequences data and shows miRNA function. Also, it links UCRs and miRNAs with the related human disorders and genomic properties. The current release contains over 2000 sequences from three species (human, mouse and rat). As a web application, UCbase & miRfunc is platform independent and it is accessible at <http://microrna.osu.edu/UCbase4>.

INTRODUCTION

UCbase & miRfunc is a database of (i) human, mouse and rat ultraconserved elements and (ii) microRNAs (miRNAs).

The database has three main functions:

- (i) It provides ultraconserved sequence data, annotation, secondary structure and information about the disorder related to the band. Furthermore, it

shows references and links to other resources for all UCRs published by Bejerano *et al.* (1).

- (ii) It provides miRNA sequence data, annotation, secondary structure, information about disorder related to the band, references and links to other resources for all published miRNAs in the genomes of human, mouse and rat. Primary features of the nomenclature scheme, sequences and genomics coordinates are retrieved from miRBase (2).
- (iii) It provides information about miRNAs and ultraconserved elements function and expression profiles.

This is made possible by a few automated Perl scripts linked to miRBase (<http://microrna.sanger.ac.uk>) (2), NCBI (<http://www.ncbi.nlm.nih.gov>) (3) and UCSC server (<http://genome.ucsc.edu>) (4).

ULTRACONSERVED SEQUENCES

Ultraconserved elements (UCRs) were discovered in 2004 by Bejerano and colleagues by bioinformatics comparisons of the genomes of mouse, rat and human (1). Four hundred and eighty-one UCR sequences are 200–779 bp in length showing 100% identity among the three species. Some of them contain protein coding sequences, but over half are not predicted to codify any protein (1).

Previous studies have suggested an important role for these noncoding sequences both in promoting the expression of several genes and in regulation of alternative splicing (5). Probably many of the UCRs date from a very early period in vertebrates evolution, as they have no orthologous counterparts in sea squirts, flies or worms

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even if at least one group of these UCRs evolved from a novel retrotransposon family that was active in lobe-finned fishes, and is still active today in the ‘living fossil’ Coelacanth (*Latimeria chalumnae*), the ancient link between marine and land vertebrates (5).

Recently, Calin *et al.* (6) identified a functional role for miRNAs in the transcriptional regulation of cancer-associated UCRs. They proved in tumors that differentially expressed UCRs could alter the functional characteristics of malignant cells. By combining this data with the much more elaborate model involving miRNAs in human tumorigenesis, they propose that alteration in both coding and noncoding RNAs cooperate in the initiation and/or progression of malignancy.

miRNAs

miRNAs were first described in 1993 by Lee and colleagues (7), yet the term ‘microrna’ was only introduced years later in 2001 in an article published in *Science* (8). Findings over the past 5 years supported a role for miRNAs in the regulation of crucial processes such as cell proliferation (9), apoptosis (10), development (11), differentiation (12) and metabolism (13). Each miRNA is supposed to target several hundreds of transcripts (14), making miRNAs one of the main genome regulators.

miRNAs regulate their targets by direct cleavage of the mRNA and by inhibition of protein synthesis, depending on the degree of complementarities with their target 3'-UTR regions (15). miRNAs are processed from primary transcripts known as pri-miRNAs (16) but not translated into protein. A portion of this primary transcript is recognized and cleaved by the enzyme Drosha into a miRNA precursor (pre-miRNA) (17) and finally processed to functional miRNA.

These pre-miRNAs are then processed to mature miRNAs in the cytoplasm by interaction with the endonuclease Dicer, which also initiates the formation of the RNA-induced silencing complex (RISC) (18). This complex is responsible for the gene silencing observed due to miRNA expression and RNAs interference. The pathway in plants varies slightly due to their lack of Drosha homologs (19).

At the time of this writing, the miRBase server version 11 contains 678 human pre-miRNAs (48–150 nt long) and 847 mature miRNAs (17–28 nt long).

Pre-miRNAs do not have a perfect double-stranded RNA structure and they are topped by a terminal loop (hairpin shape). Most of them are conserved between classes, the free energy is often less than –25 Kcal/mol, the GC-Ratio is usually 30–70% and the entropy is between 0 and 2 (20).

Recently, miRNA expression has been linked to cancer. The first evidence came from the finding that miR-15a and miR-16-1 are downregulated or deleted in most patients with chronic lymphocytic leukemia (CLL) (21). Several other groups have then studied the miRNA expression in cancer patients and found that miRNAs are differentially expressed in normal and tumor tissues (22–26) and, in some cases, are associated with prognosis (27).

QUERIES

The UCbase & miRfunc database contains three principal types of search:

- (i) *Multiple queries* can be performed typing the names of selected miRNAs or UCRs and obtaining as output the band, the gene name, the coordinates, the human diseases (28) related to the band and the secondary structure performed using UNAFold (29) (Figure 1). These tables can be also downloaded in Excel format. A complete list of UCRs and miRNAs gene names is available clicking on the link above the multiple queries text box.
- (ii) *miRNA and UCRs information* can also be retrieved searching for band, human Mendelian inheritance disorders (28) related to that band or just typing the name of a gene (Figure 2).
- (iii) *The use of an interactive map of biological systems and diseases* allows to link miRNAs and ultraconserved sequences to the related disorders (Figure 2).

FUNCTION

One of the most useful peculiarities of UCbase & miRfunc is that it provides tables showing ultraconserved elements properties (enhancer activity, alternative splicing, splicing NMD regulation and transcription evidence) and miRNA ‘experimentally proved’ function, addressing a number of common questions pertaining to these noncoding RNA classes (Figure 3). We decided to show the experiments that provide molecular biology evidence of a particular miRNA function. Paper references supplying only miRNA data were not included in the table.

All of this information is periodically retrieved (every 2 months) using a Perl scripts implemented with WWW::Search::Pubmed (<http://search.cpan.org/~gwilliams/WWW-Search-PubMed-1.004/>) and WWW::Mechanize (<http://search.cpan.org/dist/WWW-Mechanize/>) modules which provide an API search engine linked to the Pubmed database (30).

SEQUENCE COMPARISON

The sequence comparison page allows the researchers to match selected sequences against miRNAs and ultraconserved elements (exact/500/1000/2000/5000/10 000 bp up/downstream) (Figure 4) using The Basic Local Alignment Search Tool (BLAST) which finds regions of local similarity between sequences (31).

The researchers can also align miRNAs and ultraconserved elements versus TRANSFAC (32) and CpG Island Searcher databases (33). Checking the parsing option it is possible to turn the output into a single-line summary. This function is invoked internally by a Perl script which uses the Bio::SearchIO module (<http://search.cpan.org/~birney/bioperl-1.2.3/Bio/SearchIO.pm>).

Moreover it is possible to download a RepeatMasker (34) miRNAs/UCRs table showing the repetitive miRNAs and ultraconserved sequences in human genome.

UCbase & miRfunc

ULTRACONSERVED SEQUENCES AND MiRNA FUNCTION DATABASE

Multi Search function (?)

uc mir

uc.1
uc.77A

Submit Query **Reset**

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UCbase info

UCbase and MiRFunc version 1.0 Released

Ultraconserved database at microrna.org/ucrdb

Description: UCbase & MiRfunc is a database of (i) human microRNAs and (ii) Ultraconserved elements providing information about function, expression and correlation between these classes of non-coding RNAs and the disorders related to their aberrant expression.

Background: 481 Ultraconserved sequences (UCRs) longer than 200 bases were discovered in the genomes of human, mice and rats. These are DNA sequences showing 100 percent identity among the human, mouse and rat genomes. UCRs are frequently located at genomic regions involved in cancer, differentially expressed in human leukemias and carcinomas and in some instances regulated by microRNAs (miRNAs), the most extensively studied category of non-coding RNAs (ncRNAs). Here we present the first database which links UCRs and miRNAs with the related human disorders and genomic properties.

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Web SSH Login

Username
Password
Login

Figure 1. Multi-queries text box (red square). Multiple queries can be performed typing the names of selected miRNAs or UCRs and obtaining as output the band, the gene name, the coordinates, the human diseases related to the band and the secondary structure up to 500 bp upstream/downstream.

EXPRESSION

UCbase & miRfunc database is linked to microrna.org (35) to retrieve miRNA gene expression data sets; whereas, it collects ultraconserved sequences microarray expression data from ArrayExpress (36), GEO (37) and microrna.osu.edu server.

DATABASE INTERFACE

Cascading Style Sheets (CSS) were used for UCbase & miRfunc web development to style web pages written in HTML. The CSS specifications are maintained by the World Wide Web Consortium (W3C). Internet media type (MIME type) text/css is registered for use with CSS by RFC 2318 (March 1998).

UCbase & miRfunc was created using MySQL database under Debian Etch Linux OS installed on a Quad Core Processor machine with 32 GB RAM.

UCbase & miRfunc database can be accessed by Perl programs running on the web server. The MySQL Perl API is mainly provided with the use of CGI (Common Gateway Interface) which is a standard for external gateway programs to interface with information servers such as HTTP servers (Apache2 in this particular case). A CGI object (cgi.pm) was used to handle POST and GET methods correctly, and distinguish between scripts called from ISINDEX documents and form-based documents.

DATA SOURCES AND IMPLEMENTATION

Almost all the code used to develop the database and obtain miRNAs and ultraconserved elements information was written in Perl.

Perl (www.perl.org) is a programming language originally created for text manipulation and for a wide range of tasks including system administration, web development,

UCbase & miRfunc

ULTRACONSERVED SEQUENCES AND miRNA FUNCTION DATABASE

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Human Chromosome Location

miRNA and UC signature in cancer and type of disease

System or Type of Disease			
Cancer	Nervous	Muscle	Immune
Metabolism	Signals	Transport	

miRNA Search by band or OMIM disorder

1p13.1 17,20-lyase deficiency, isolated

Search by GENE (Ex. DLEU2 or intergenic)

UC Search by band or OMIM disorder

1p13.1 17,20-lyase deficiency, isolated

Search by GENE (Ex. PEX14 or NA)

Help

miRNA and Ultraconserved sequences Searching : How to...

This database is constructed allowing the user to find microRNA or ultraconserved sequences searching them by band, by gene or by OMIM disorder correlated to that band.

Figure 2. Search Page. miRNA and UCRs information can be retrieved searching for band, human Mendelian inheritance disorders related to that band or just typing the name of a gene (red highlighted boxes). The use of an interactive map of biological systems and diseases allows to link miRNAs and ultraconserved sequences to the related disorders (blue arrow).

network programming and GUI development. It is one of the most common language programming in bioinformatics, where it is valued for rapid application development and deployment, and the ability to handle large data sets. Perl is also used for the web automation.

Web automation can automate all the web processes from simple filling of forms to more complicated tasks for data transfer, web data extraction, image recognition and performing tasks based on it, scheduling tasks, batch process and file management. A Perl module called

UC function Table															
id	name	ultra conserved element		upstream gene	within	downstream gene	Human	Location	RNAfold	p-value					
		name	type								length	Build 34 (hg16) coords	distance	name	gene
9	mir-9	uc.1	p	207	chr1:10307243-10307449	65151	DFFA	AK130996	99286	0	0	1p36.32	na	0.0983	
		uc.2	n	207	chr1:10442089-10442295	34690	AK130996	FLJ20321	273783	0	0	1p36.32	na	0.0707	
		uc.3	p	225	chr1:10460711-10460935	53312	AK130996	FLJ20321	255143	0	0	1p36.32	na	0.1018	
		uc.4	n	359	chr1:10467795-10468153	4286	FLJ20321	'N	FLJ37118	247925	0	0	1p36.32	na	0.4531
		uc.5	n	214	chr1:10490897-10491110	27380	FLJ20321	'N	FLJ37118	224968	0	0	1p36.32	na	0.0574
		uc.6	p	301	chr1:10504667-10504967	41150	FLJ20321	'N	FLJ37118	211111	0	0	1p36.32	na	0.2363
		uc.7	p	256	chr1:10545679-10545934	82170	FLJ20321	'N	FLJ37118	170144	0	0	1p36.32	na	0.2663
		uc.8	n	216	chr1:10561364-10561579	97855	FLJ20321	'N	FLJ37118	154499	0	0	1p36.32	na	0.2265
		uc.9	n	202	chr1:10634956-10635152	171447	FLJ20321	'N	FLJ37118	80921	0	0	1p36.32	na	0.0222
		uc.10	p	275	chr1:10675120-10675394	211611	FLJ20321	'N	FLJ37118	40684	0	0	1p36.32	na	0.0177
		uc.11	p	201	chr1:35077889-35078089	60766	SFPQ	ZNF262	140539	0	0	1p34.3	Galbladder disease 3 (2)	0.6009	
		uc.12	e	237	chr1:35786852-35787088	36769	EIF2C4	EIF2C3	36892	137	137	1p34.3	Galbladder disease 3 (2)	0.1805	
		uc.13	p	213	chr1:37900298-37900510	5531	CGI-94	'N	POU3F1	15061	0	0	1p34.3	Galbladder disease 3 (2)	0.3971
		uc.14	n	233	chr1:37974370-37974602	48625	POU3F1	'N	RRAGC	742565	0	0	1p34.3	Galbladder disease 3 (2)	0.4661
		uc.15	p	211	chr1:38041493-38041703	115748	POU3F1	'N	RRAGC	675464	0	0	1p34.3	Galbladder disease 3 (2)	0.0929
		uc.16	n	237	chr1:38215445-38215601	289700	POU3F1	'N	RRAGC	501486	0	0	1p34.3	Galbladder disease 3 (2)	0.3543
		uc.17											Methylmalonic aciduria and homocystinuria, cbC type,		

Figure 3. Function page. It provides tables showing ultraconserved elements properties (enhancer activity, alternative splicing, splicing NMD regulation and transcription evidence) and miRNA ‘experimentally proved’ function.

WWW::Mechanize was used to extract the data from NCBI (search_pubmed_novo.pl) and miRBase (www_mechanize_sanger_total.pl) pages; whereas, a script written in Pascal (<http://www.newbie.com>) was used to extract miRNAs and ultraconserved sequences (500/1000/2000/5000/10 000 bp up/downstream) from UCSC server (ucsc.nbl).

In particular, all the utilities that come with WWW::Mechanize print the names and elements of every form and provide all the needed information when searching for form using regular expressions (<http://www.opengroup.org/onlinelpubs/007908799/xbd/re.html>).

However, using that data in the code means to cut and paste multiple entire blocks of code. For this reason, it is useful to install HTTP::Recorder to set up an HTTP proxy. HTTP::Recorder saves each action as WWW::Mechanize code. Before running the recording script (recorder.pl), the browser proxy has to be

configured properly. Running recorder.pl starts an HTTP proxy daemon that the browser uses to make requests. The proxy uses HTTP::Recorder agent to log these requests. It saves a logfile as a .t file, which is specified when creating the HTTP::Recorder object.

Moreover, a window will appear displaying the content of .t file which should be a series of statements involving a hypothetical WWW::Mechanize object. These scripts were added to the final Perl code file called www_mechanize_sanger_total.pl.

WWW::Search::PubMed version 1.004 provides instead a backend to the WWW::Search module allowing searches of the National Library of Medicine’s PubMed biomedical citation database (30) (pubmed.pl).

All the miRNAs genomic information are obtained using an automatic script linked to miRBase ftp server (ftp://ftp.sanger.ac.uk/pub/mirbase/sequences/CURRENT/database_files) which runs periodically

Multi Search function (?)

uc mir

Search for sequence or name UCR

Submit Query Reset

UCbase Download

Download db and sequences

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Figure 4. Sequence comparison page. This page allows the researchers to match selected sequences against miRNAs and ultraconserved elements (exact/500/1000/2000/5000/10 000 bp up/downstream). Checking the parsing option it is possible to turn the output into a single-line summary (blue arrow).

using CRON, a time-based scheduling service in Unix-like computer operating systems (<http://packages.debian.org/etch/cron>).

CONCLUSIONS

UCbase & miRfunc is the first database containing the long 481 ultraconserved sequences discovered in the genomes of human, mouse and rat by Bejerano *et al.* (1) and identified as deregulated in cancer by Calin *et al.* (6). In addition, UCbase & miRfunc is the first database that provides miRNA function, which is a particularly important feature due to the increasing output of miRNA data. The goal of this study is to offer the researchers an advanced set of tools that supply information about correlation between miRNAs, UCRs and the disorders related to their aberrant expression.

The first version of UCbase & miRfunc includes new methods of queries such as search for band, disorders and genes. It has the capability of using multiple queries and combining the results from several miRNA/UCRs input. In addition, the system has a new tool for the RNA secondary structure prediction (up to 500 bp up/downstream), which allows innovative visualization

of miRNAs and ultraconserved elements. This feature improves presentation of data output that is especially useful when several structures are obtained using multiple queries.

The sequence comparison tool, which is also available, allows researchers to match selected sequences that are 500/1000/2000/5000/10 000 bp upstream/downstream of all defined miRNAs and UCRs in the database.

Additionally, UCbase & miRfunc has adopted several strategies to integrate search tools. It provides updated data by using automate web scripts.

The system will be regularly upgraded with new human, mouse and rat miRNAs/UCRs by providing the corresponding reference sequences and annotations, thus allowing the data to be refined continuously with every new miRBase and UCSC database version. It also provides complete human, mouse and rat miRBase table as a single file (available from the resource web page), a feature that is particularly useful if one wants to use regular expressions to identify the segment of the input file associated with sub-field of interest.

Although other alternatives are available for retrieval of miRNA nomenclature, sequence data and annotation, UCbase & miRfunc is unique combination of features that provide biologists many options for data analysis

and discovery of relationships between miRNAs and ultraconserved sequences.

FUTURE DIRECTIONS

The improvements to a new set of OMIM (28) and HGMD (38) databases needed for an overall plan for collection (39), hopefully should enable UCbase & miRfunc users, in the next year, to fully exploit the relation between miRNAs/UCRs and the mutation or chromosome aberrations related to their genomic location.

SUPPLEMENTARY DATA

Supplementary data and programming codes are available at <http://web.unife.it/utenti/cristian.taccioli/software/>. The user has to rename the codes files into .pl format because of security reasons we have uploaded them as .txt.

Free web tools written for miRNA and UCRs microarray analysis can also be downloaded at <http://web.unife.it/utenti/cristian.taccioli/mix/examples.html>.

ACKNOWLEDGEMENTS

We gratefully acknowledge the support of all members of the Department of Molecular Virology, Immunology and Medical Genetics of The Ohio State University. We also appreciate the technical support of Melissa Dickman, Daniela Taccioli, Ivan Tassani, Stefan Costinean, Nicola Zanesi, Pierluigi Gasparini and Gianpiero Di Leva.

FUNDING

Funding for open access charge: OSU Human Cancer Genetics Program.

Conflict of interest statement. None declared.

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